Identification of Yeasts

Medical Mycology Training Network 15 November 2018
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Definition of Yeasts

- Eukaryote – cells have defined nucleus and nuclear membrane
- Single cell (vs mould mostly multicellular and forms mycelium) that reproduces by budding
- Reproduce by sexual and asexual process

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Commonly isolated yeasts in humans

- Candida
- Cryptococcus
- Trichosporon
- Saccharomyces
- Rhodotorula
- Geotrichum
- Malassezia

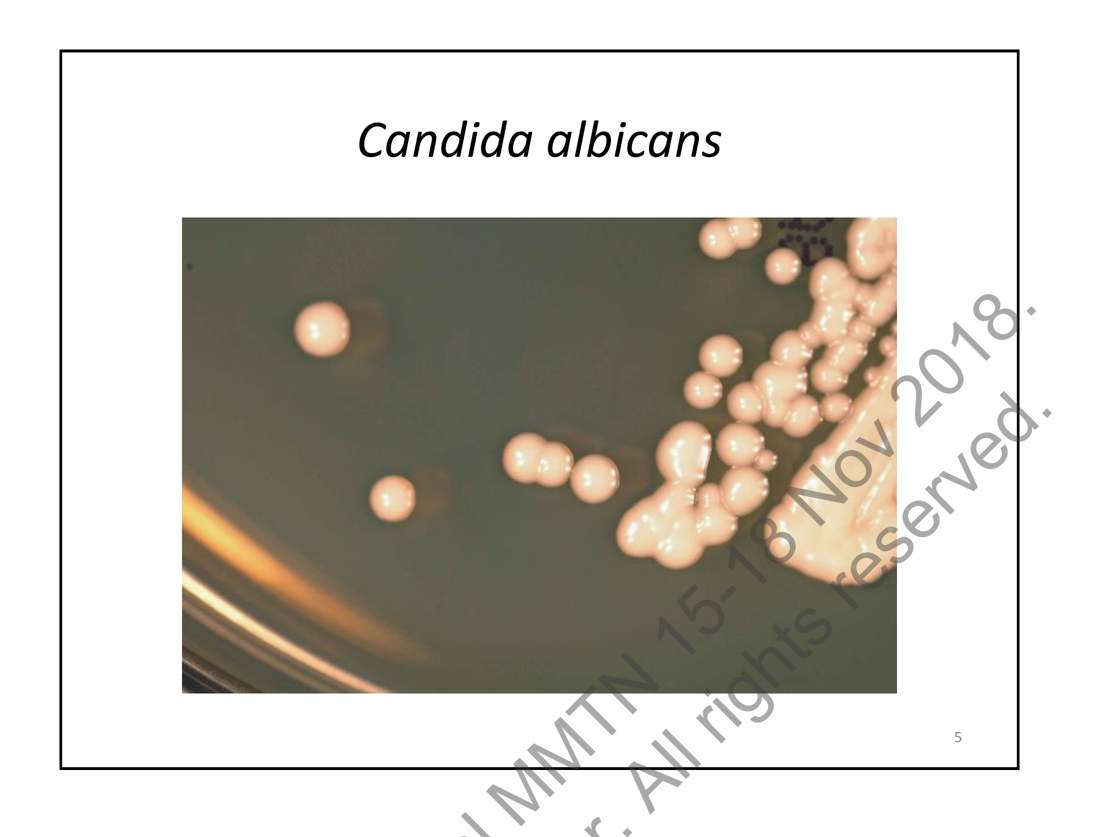
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- Yeasts – appearance is generally moist looking with smooth round colonies
- Moulds – appearance is rough, dry colonies and may see aerial hyphae

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Candida albicans



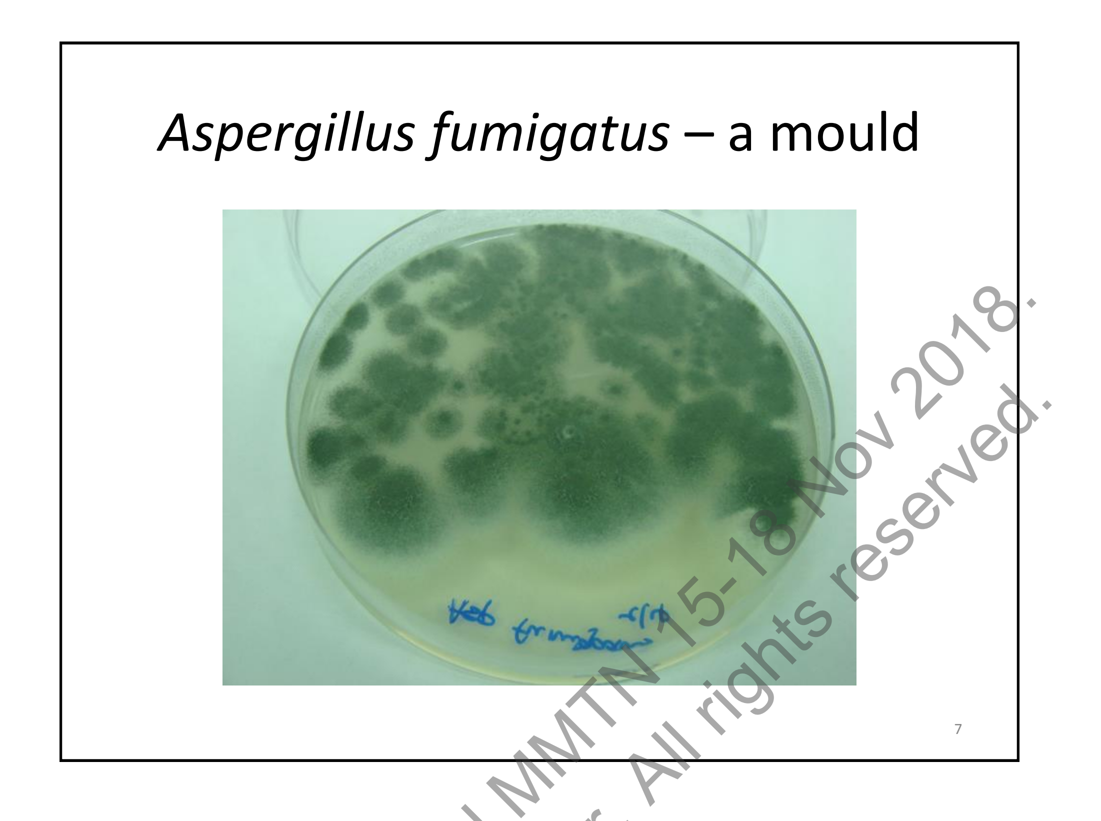
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Candida albicans
(wet mount)

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Aspergillus fumigatus – a mould



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Aspergillus fumigatus – tease mount

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Steps for yeast identification

- Gross morphology
- Microscopic morphology (wet mount)
- Yeast morphology (Dalmau plate) and urease test
- Germ tube
- Biochemical tests (API, Vitek etc)
- MALDI-TOF
- Other tests that may be used: Temperature (35^0C, 42^0C), Nitrate utilisation, Cycloheximide resistance
- Molecular sequencing may be used for some cases

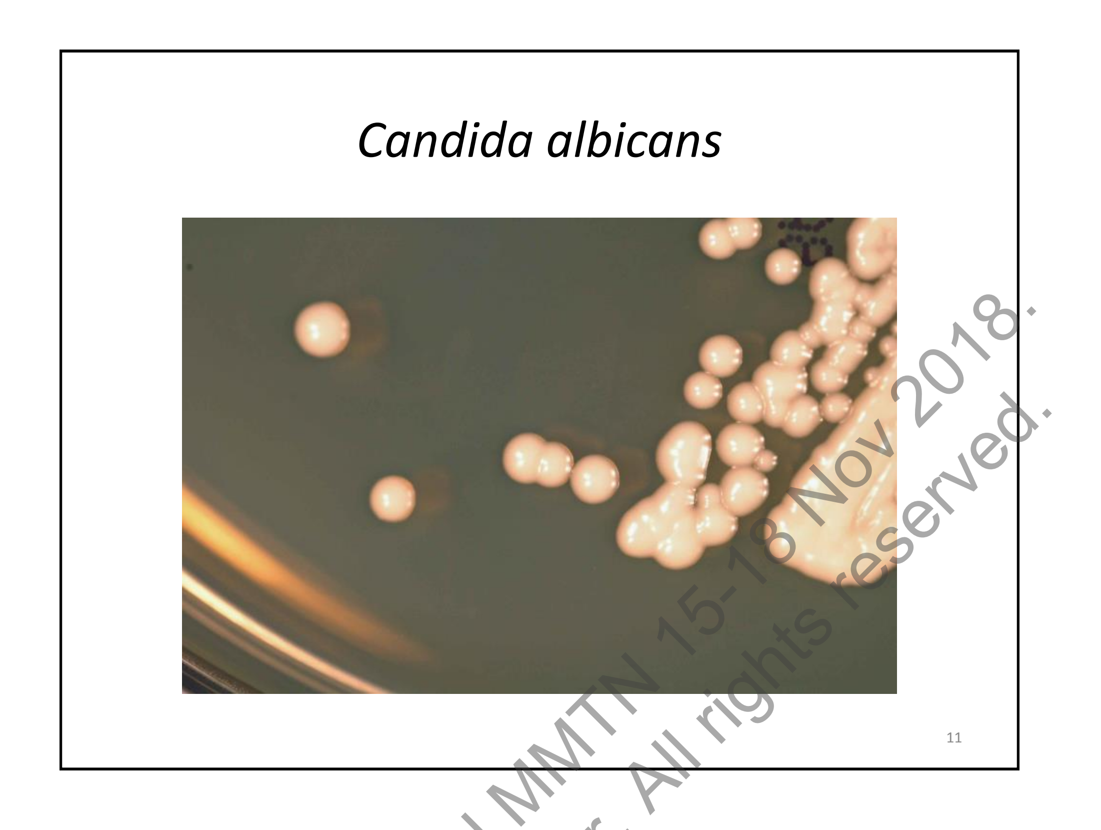
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Identification of yeasts (1)

- Starts with gross morphology – plated on media (eg Sabouraud dextrose agar)
- Appearance on plated media
- Colour
- Texture (mucoid etc)

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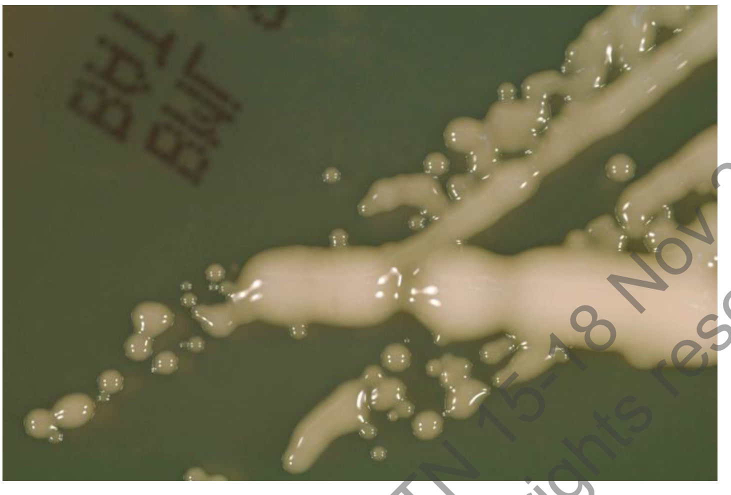
Rhodotorula

Pinkish colonies

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Cryptococcus neoformans



Mucoid colonies

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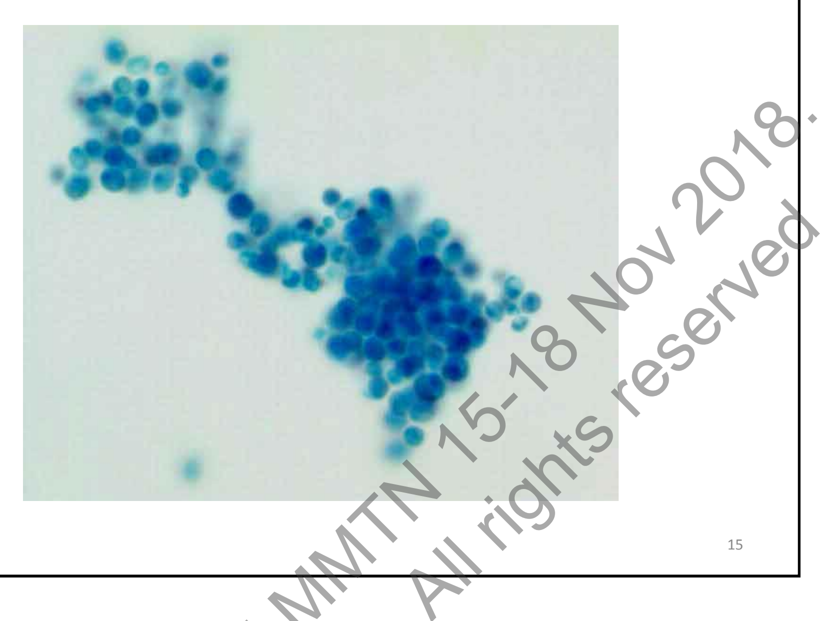
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Identification of yeasts (2)

- Do microscopic examination - perform a wet mount

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Candida albicans
(wet mount with lactophenol cotton blue)



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Identification of yeasts (3)

- Perform yeast morphology. Use Cornmeal agar with 1% Tween 80 (Dalmau plate). Originally meant to demonstrate presence of chlamydospore (chlamydoconidia) – tentatively identifies as Candida albicans. Other features seen include blastoconidia, pseudohyphae, hyphae, arthroconida.
- Can narrow down to genus level.

Yeast morphology using Dalmau plate

- Summarised procedure:
- Scratch a light inoculum into the surface of agar (2 streaks about 2cm long and 1cm apart, and repeat 2 streaks perpendicular to first set of streaks), and place a clean coverslip over the area.
- Incubate at 22-26^{0}C for 48 hours, or longer if necessary.
- Remove the petri dish cover, and examine the plate under low power, focus on edge of coverslip then scan the rest of the area for chlamydospores and other structures.

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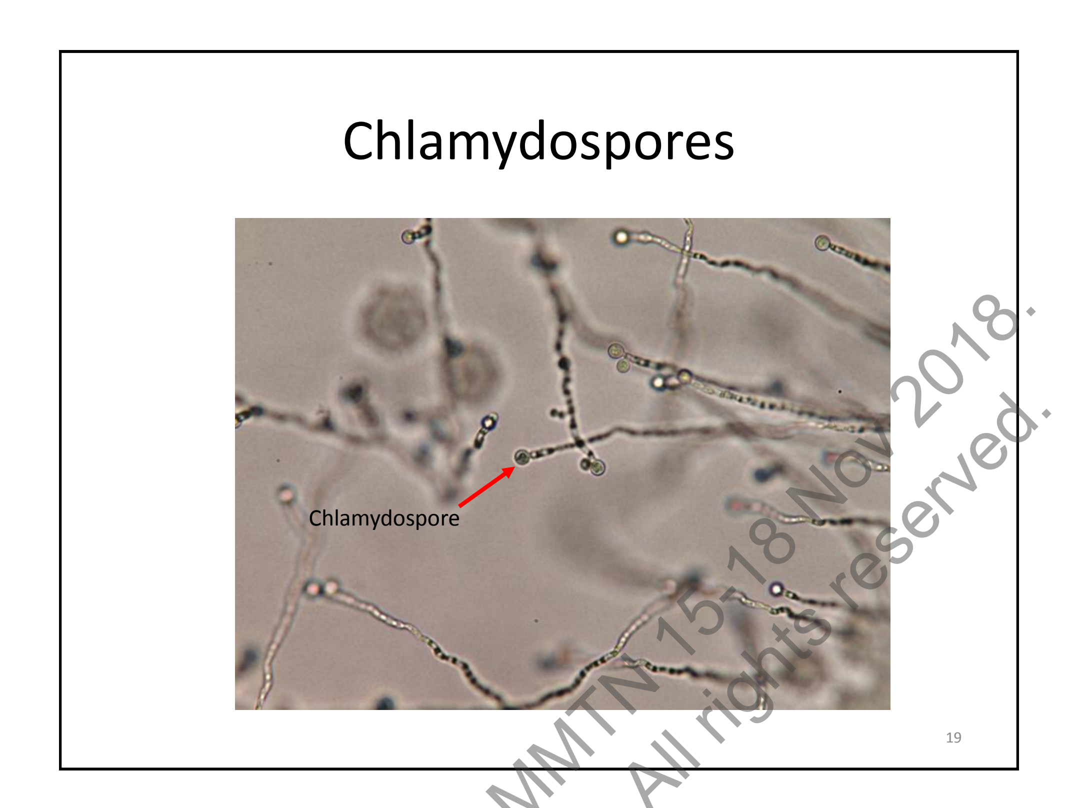
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Yeast morphology

Structures observed	Probable Identification
Pseudohyphae (occasional hyphae) Blastoconidia Chlamydospores	Candida albicans Candida dubliniensis
Pseudohyphae (occasional hyphae) Blastoconidia	Candida species
Blastoconidia	Candida species (C. glabrata) Cryptococcus species Rhodotorula species Saccharomyces species (has ascospores)
Hyphae Blastoconidia Arthroconidia	Trichosporon species
Hyphae Arthroconidia	Geotrichum species
Sporangium with sporangiospores	Prototheca (an algae)

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Chlamydospores



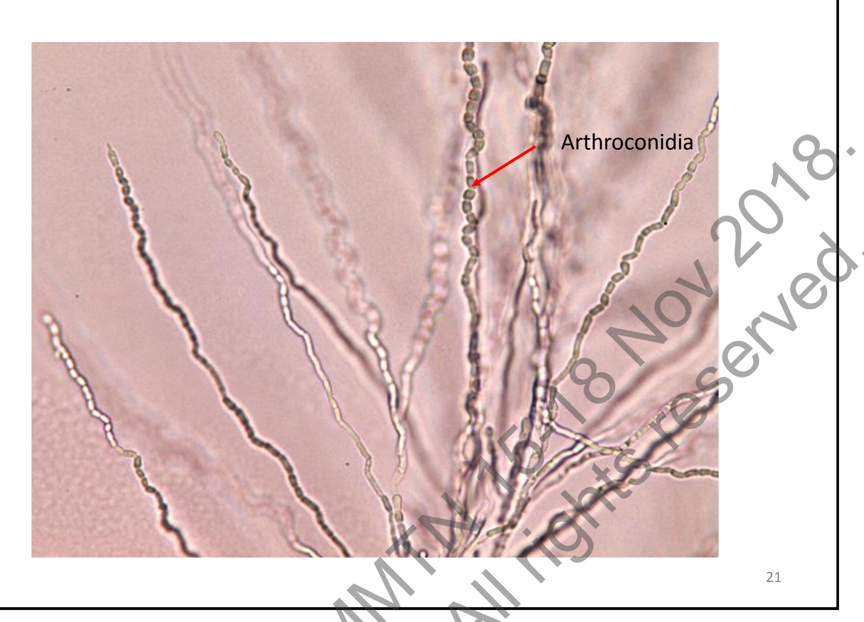
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Blastoconidia and Pseudohyphae

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Arthroconida



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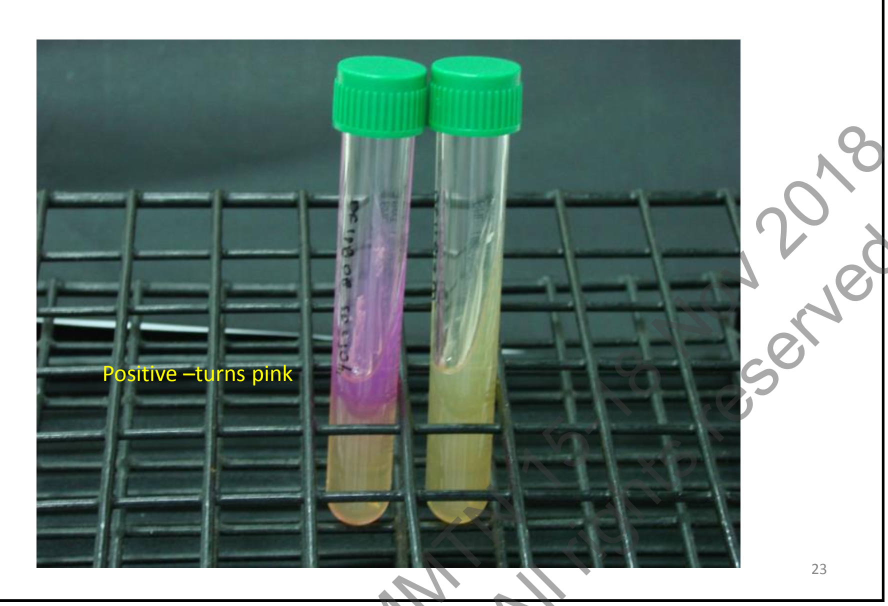
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Urea test

- Done together with yeast morphology, to help identification of yeast.
- Urea positive yeasts:
  - Cryptococcus
  - Trichosporon
  - Rhodotorula
  - Some Candida (Pichia kudriavzevii old name C. krusei, Yarrowia lipolytica old name C. lipolytica)

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Urease test



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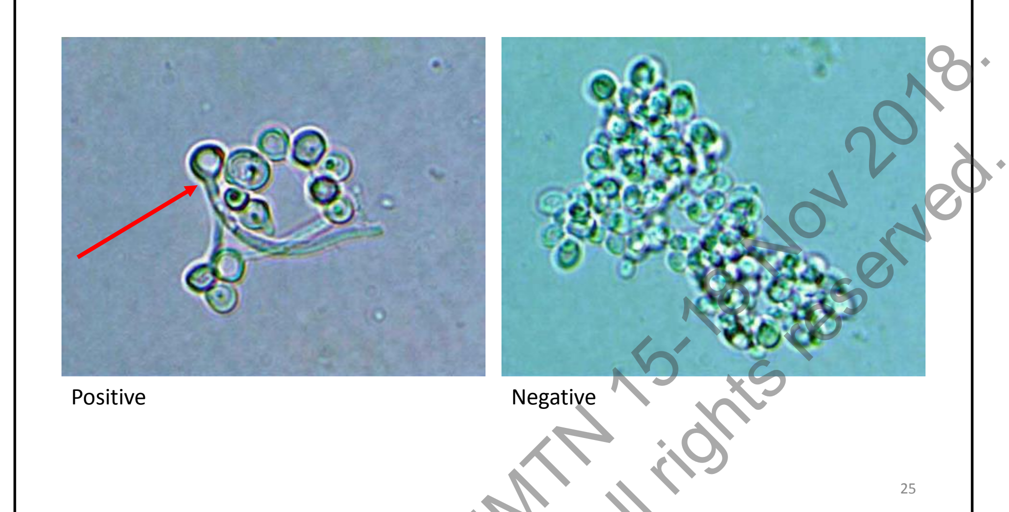
Identification of yeasts (4) – germ tube test for Candida species

- Once narrowed down to Candida species, perform germ tube test.
- Procedure – inoculate a light inoculum of yeast from a young culture into 0.5 mL serum containing 0.5% glucose, incubate at 35^{0}C for 2-3 hours. Look for tube like structures appearing from blastoconidia (no constriction at the point of formation).
- Candida albicans and dubliniensis form germ tubes. Need other tests to differentiate – eg API

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Germ tube



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Identification of Candida species

- Yeast morphology on Cornmeal Agar plate
- Urease test
- Do germ tube
- Do biochemical tests (able to identify Candida species, and some of the other yeasts)
  - API (20C AUX)
  - Vitek 2 (ID-YST)
  - Microscan (Yeast Identification Panel)
- MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight)

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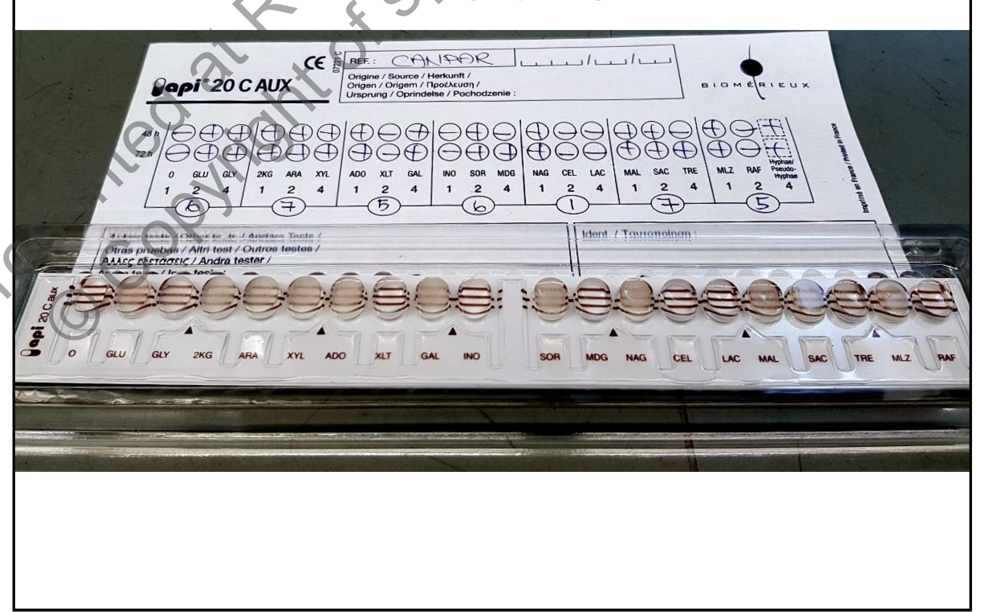
API

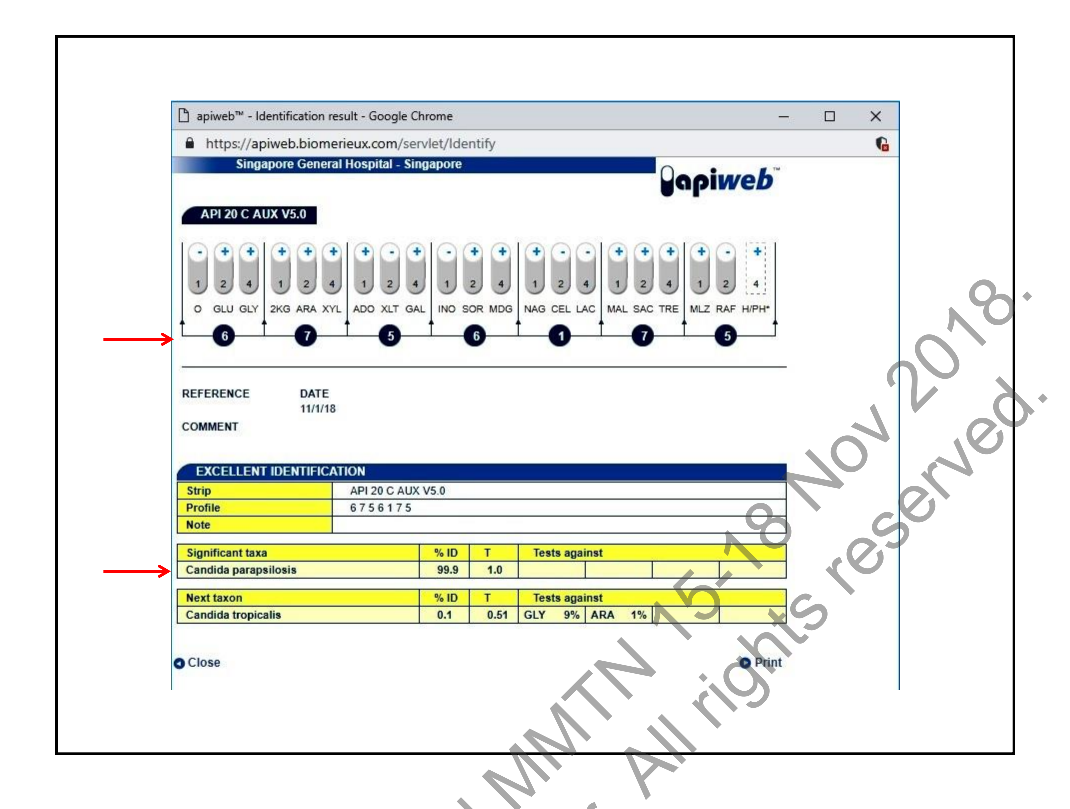
- API – 20C AUX
- A series of 20 biochemical reactions including assimilation tests, dehydrated and in cupules
- Inoculate a standardised inoculum into each of the cupule. Incubate 30°C for 48 hours, or longer. Read and determine the profile number – check against database
- Advantage – well-established and generally reliable
- Disadvantage – needs 48 hours or longer
- API 32C – has more biochemical reactions

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API 20 C AUX





Vitek

- Vitek 2
- A series of biochemical tests on a card
- Inoculate a standardised inoculum into each of the cupule. Incubate 35ºC for 24 hours. Print out the identification.
- Advantages – easy to use. Can get results in 24 hours.
- Disadvantage - not good for some yeasts eg *Trichosporon* misidentified at *Cryptococcus laurentii*. *Candida auris* misidentified as *Candida haemulonis complex.*
- Although version 8.01 and above can identify *Candida auris,* but some are still misidentified.

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Vitek 2 cards



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Vitek 2

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bioMérieux Customer: | Laboratory Report | Printed Nov 1, 2018 14:29 SGT
System #: | | Printed by: sghmicro

Patient Name: | Patient ID:
Isolate: Candida auris-1 (Qualified) | Bench: Resp

Card Type: YST Bar Code: 2430613203538513 Testing Instrument: 0000148FFC35 (SGH 2223)
Card Type: AST-YS08 Bar Code: 2880660103403348 Testing Instrument: 0000148FFC35 (SGH 2223)
Setup Technologist: ???(SGH)

Bionumber: 4150145245321771
Organism Quantity: **Selected Organism: Candida auris**

Comments:	

McFarland: **(1.80 - 2.20)**

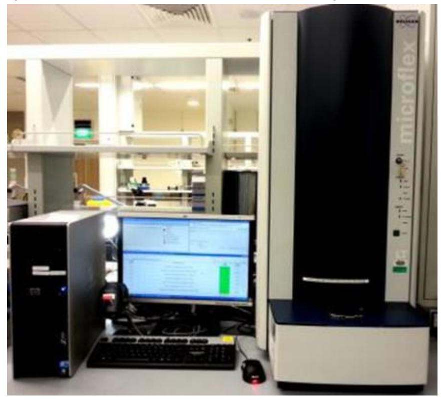
Identification Information	**Card:**	YST	**Lot Number:**	2430613203	**Expires:**	Aug 1, 2019 12:00 SGT
	Completed:	Nov 1, 2018 09:39 SGT	**Status:**	Final	**Analysis Time:**	17.85 hours
Organism Origin	VITEK 2					
Selected Organism	98% Probability		**Candida auris**			
	Bionumber:	4150145245321771			**Confidence:**	Excellent identification
SRF Organism						
Analysis Organisms and Tests to Separate:						
Analysis Messages: Preliminary - CAUTION: Confirm with final report.						
Contraindicating Typical Biopattern(s) Candida auris	ARBa(20),					

MALDI-ToF Mass Spectrometry

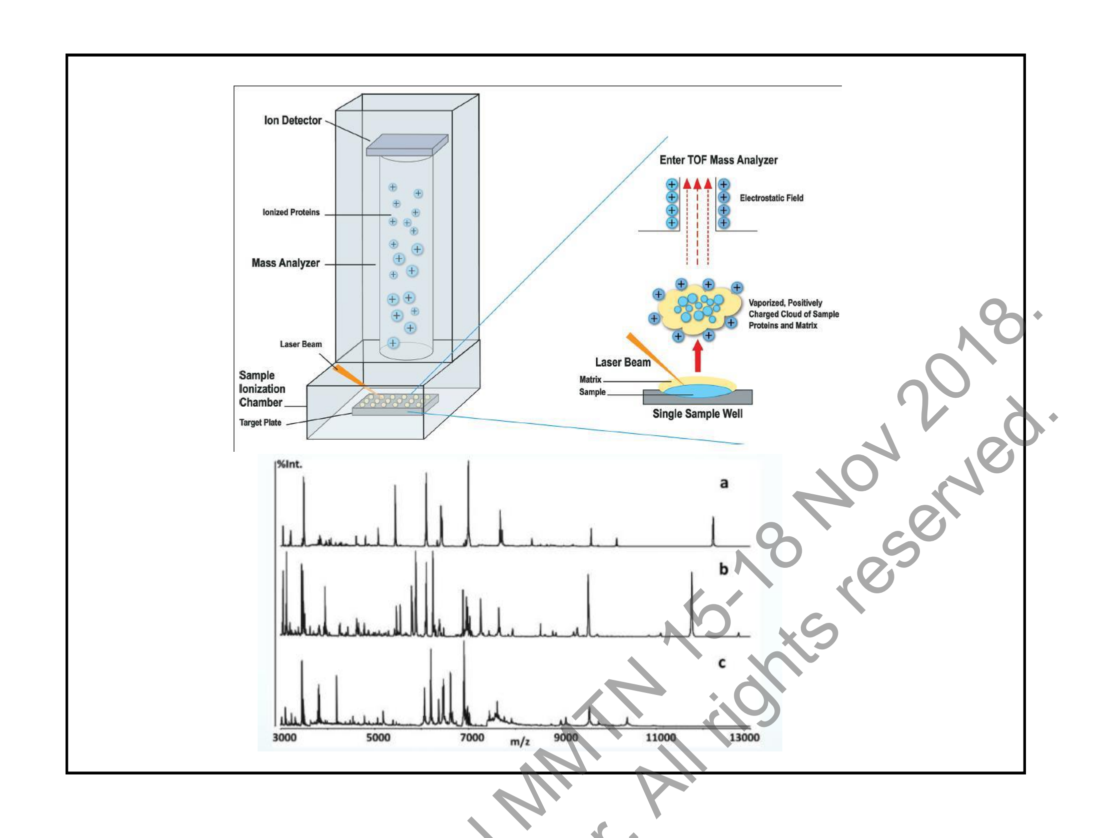
Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

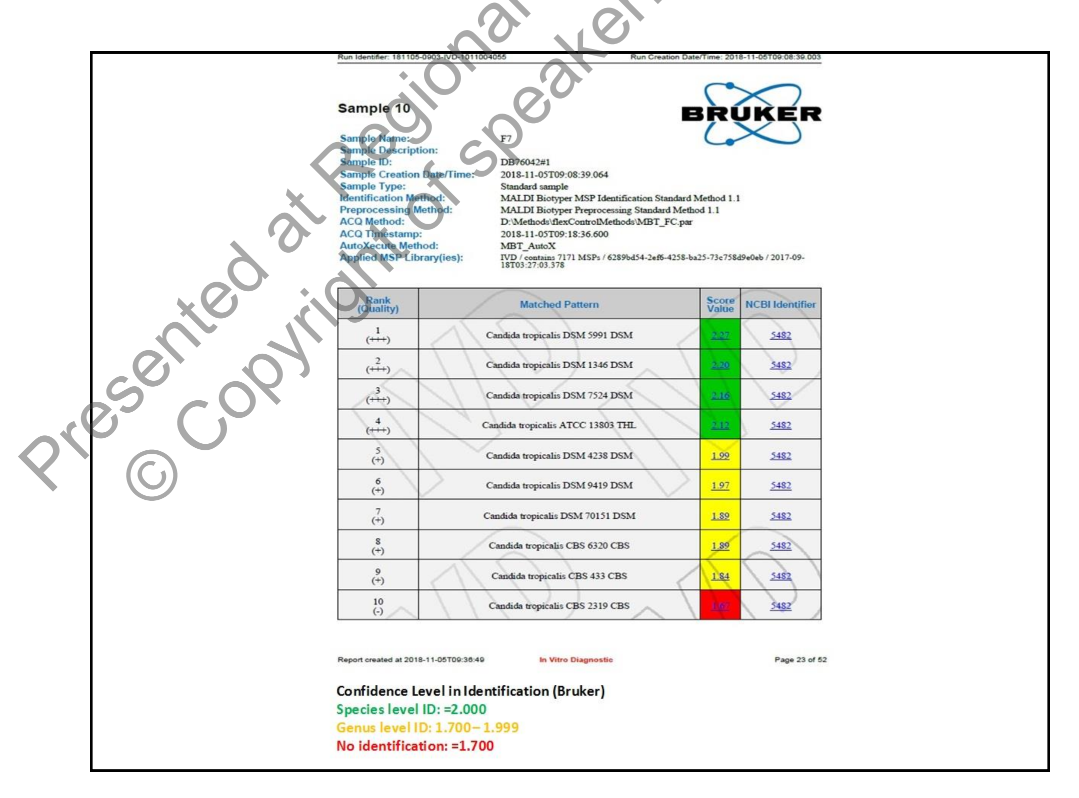
Two frequently used systems:

- MALDI Biotyper CA System (Bruker Daltonics Inc.)
- Vitek MS (bioMerieux Inc.)



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Run Identifier: 181105-0903-IVD-1011004055 Run Creation Date/Time: 2018-11-05T09:08:39.003

Sample 10

BRUKER

Sample Name: F7
Sample Description:
Sample ID: DB76042#1
Sample Creation Date/Time: 2018-11-05T09:08:39.064
Sample Type: Standard sample
Identification Method: MALDI Biotyper MSP Identification Standard Method 1.1
Preprocessing Method: MALDI Biotyper Preprocessing Standard Method 1.1
ACQ Method: D:\Methods\flexControlMethods\MBT_FC.par
ACQ Timestamp: 2018-11-05T09:18:36.600
AutoXecute Method: MBT_AutoX
Applied MSP Library(ies): IVD / contains 7171 MSPs / 6289bd54-2ef6-4258-ba25-73c758d9e0eb / 2017-09-18T03:27:03.378

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Candida tropicalis DSM 5991 DSM	2.27	5482
2 (+++)	Candida tropicalis DSM 1346 DSM	2.20	5482
3 (+++)	Candida tropicalis DSM 7524 DSM	2.16	5482
4 (+++)	Candida tropicalis ATCC 13803 THL	2.12	5482
5 (+)	Candida tropicalis DSM 4238 DSM	1.99	5482
6 (+)	Candida tropicalis DSM 9419 DSM	1.97	5482
7 (+)	Candida tropicalis DSM 70151 DSM	1.89	5482
8 (+)	Candida tropicalis CBS 6320 CBS	1.89	5482
9 (+)	Candida tropicalis CBS 433 CBS	1.84	5482
10 (-)	Candida tropicalis CBS 2319 CBS	1.67	5482

Report created at 2018-11-05T09:36:49 In Vitro Diagnostic Page 23 of 52

Confidence Level in Identification (Bruker)

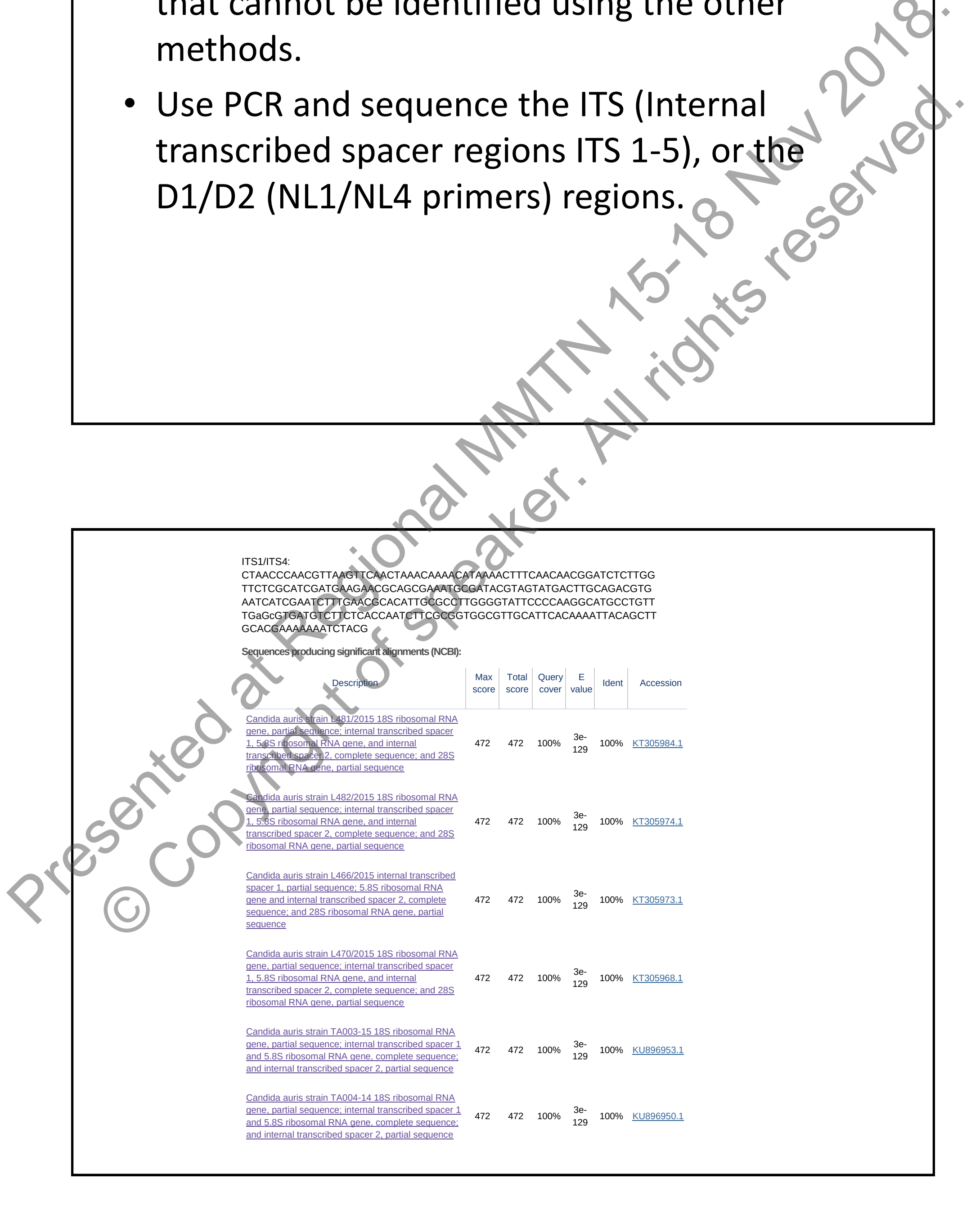
Species level ID: =2.000
Genus level ID: 1.700 – 1.999
No identification: =1.700

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Molecular sequencing

- Used for rare species of Candida and yeasts that cannot be identified using the other methods.
- Use PCR and sequence the ITS (Internal transcribed spacer regions ITS 1-5), or the D1/D2 (NL1/NL4 primers) regions.

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ITS1/ITS4:
CTAACCCAACGTTAAGTTCAACTAAACAAAACATAAAACTTTCAACAACGGATCTCTTGG
TTCTCGCATCGATGAAGAACGCAGCGAAATGCGATACGTAGTATGACTTGCAGACGTG
AATCATCGAATCTTTGAACGCACATTGCGCCTTGGGGTATTCCCCAAGGCATGCCTGTT
TGaGcGTGATGTCTTCTCACCAATCTTCGCGGTGGCGTTGCATTCACAAAATTACAGCTT
GCACGAAAAAAATCTACG

Sequences producing significant alignments (NCBI):

Description	Max score	Total score	Query cover	E value	Ident	Accession
Candida auris strain L481/2015 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	472	472	100%	3e-129	100%	KT305984.1
Candida auris strain L482/2015 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	472	472	100%	3e-129	100%	KT305974.1
Candida auris strain L466/2015 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	472	472	100%	3e-129	100%	KT305973.1
Candida auris strain L470/2015 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	472	472	100%	3e-129	100%	KT305968.1
Candida auris strain TA003-15 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	472	472	100%	3e-129	100%	KU896953.1
Candida auris strain TA004-14 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	472	472	100%	3e-129	100%	KU896950.1

NL1/NL4:
TAACGGCGAGTGAAGCGGCAAGAGCTCAACTTTGGAATCGCTCCGGCGAGTTGTAGTC
TGGAGGTGGCCACCACGAGGTGTTCTAGCAGCAGGCAAGTCCTTTGGAACAAGGCGC
CAGCGAGGGTGACAGCCCCGTACCTGCTTTTGCTAGTGCTTCCTGTGGCCCACCGACG
AGTCGAGTTGTTTGGGAATGCAGCTCTAAGTGGGTGGTAAATTCCATCTAAGGCTAAAT
ATTGGCGAGAGACCGATAGCGAACAAGTACAGTGATGGAAAGATGAAAAGCACTTTGA
AAAGAGAGTGAAACAGTACGTGAAATTGTTGAAAGGGAAGGGCTTGCACCCAGACACG
GTTTTACCGGGCCAGCATGGGTTATTACAGGGTCAAAATGACCAGGGAATGTAGCTAC
CTCTTGGTAGTGTTATAGCCCTGGTTGATGGCCCTCGTGGTGACCGAGGACCGCGGTC
TCTAGGA

Sequences producing significant alignments (NCBI) :

Description	Max score	Total score	Query cover	E value	Ident	Accession
Candida auris strain TA003-14 28S ribosomal RNA gene, partial sequence	749	749	100%	0.0	95%	KU886679.1
Candida auris strain TA002-15 28S ribosomal RNA gene, partial sequence	749	749	100%	0.0	95%	KU886678.1
[Candida] auris culture DSM:21092 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	734	734	100%	0.0	95%	MF817727.1
Candida auris strain MRL293 26S ribosomal RNA gene, partial sequence	734	734	100%	0.0	95%	KJ126765.1
Candida auris strain MRL209 26S ribosomal RNA gene, partial sequence	734	734	100%	0.0	95%	KJ126763.1
Candida auris strain MRL208 26S ribosomal RNA gene, partial sequence	734	734	100%	0.0	95%	KJ126762.1
Candida sp. JHS-2008 isolate C4338 26S ribosomal RNA gene, partial sequence	734	734	100%	0.0	95%	EU881967.1
Candida sp. JHS-2008 isolate C4510 26S ribosomal RNA gene, partial sequence	734	734	100%	0.0	95%	EU881965.1
Candida sp. JHS-2008 isolate C4509 26S ribosomal RNA gene, partial sequence	734	734	100%	0.0	95%	EU881964.1
Candida sp. JHS-2008 isolate C3920 26S ribosomal RNA gene, partial sequence	734	734	100%	0.0	95%	EU881961.1
Candida sp. JHS-2008 isolate C3563 26S ribosomal RNA gene, partial sequence	734	734	100%	0.0	95%	EU881960.1
Candida sp. JHS-2008 isolate C4049 26S ribosomal RNA gene, partial sequence	734	734	100%	0.0	95%	EU881958.1

Other useful tests: “Spiking” for *Candida albicans*

- A rapid and simple method of identifying *C. albicans* by morphology approved by CLSI guidelines. *C. albicans* (and *C. dubliniensis*) form “spikes” or “feet”, whereas the other species do not.
- Improved sensitivity when incubated in CO_2 and adequate incubation time (24 to 48 hours).
- Caveat: *Trichosporon* species can also form spikes

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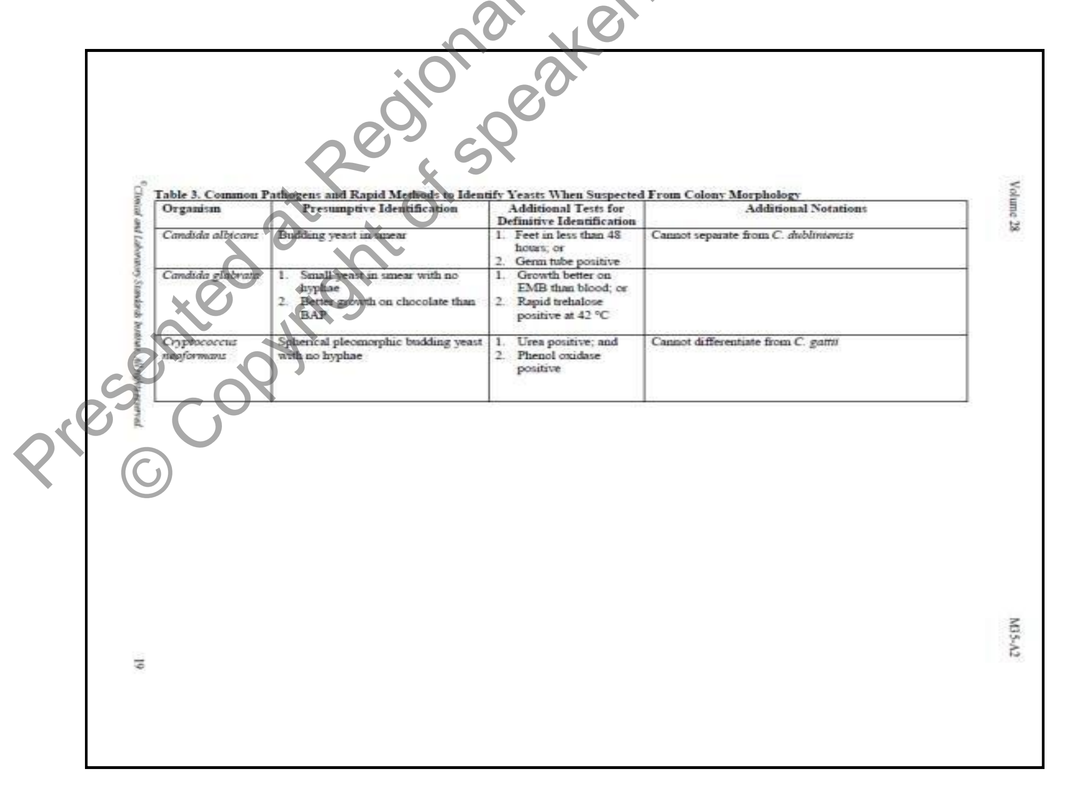


Table 3. Common Pathogens and Rapid Methods to Identify Yeasts When Suspected From Colony Morphology

Organism	Presumptive Identification	Additional Tests for Definitive Identification	Additional Notations
Candida albicans	Budding yeast in smear	1. Feet in less than 48 hours; or 2. Germ tube positive	Cannot separate from *C. dubliniensis*
Candida glabrata	1. Small yeast in smear with no hyphae 2. Better growth on chocolate than BAP	1. Growth better on EMB than blood; or 2. Rapid trehalose positive at 42 °C	
Cryptococcus neoformans	Spherical pleomorphic budding yeast with no hyphae	1. Urea positive; and 2. Phenol oxidase positive	Cannot differentiate from *C. gattii*

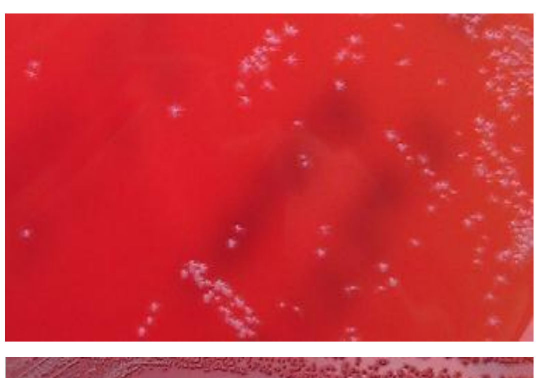
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“Spiking” appearance

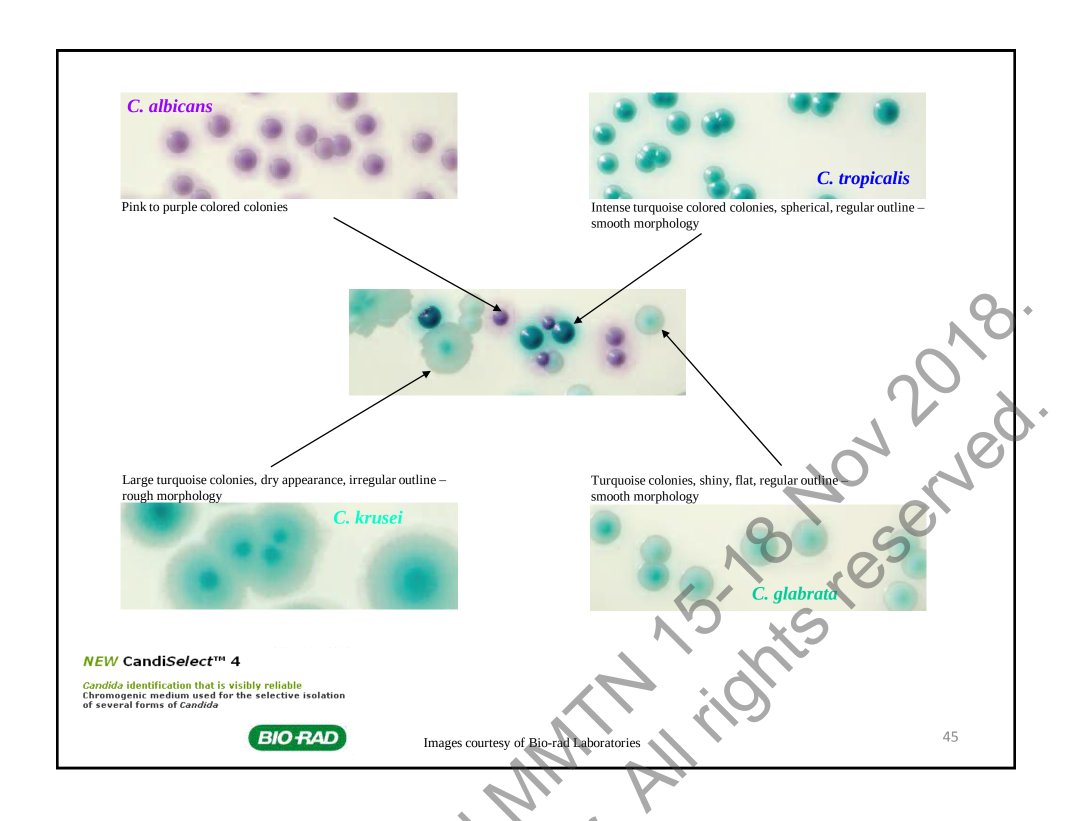
Candida albicans

Trichosporon species

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Other useful tests for Candida: Use of chromogenic agar

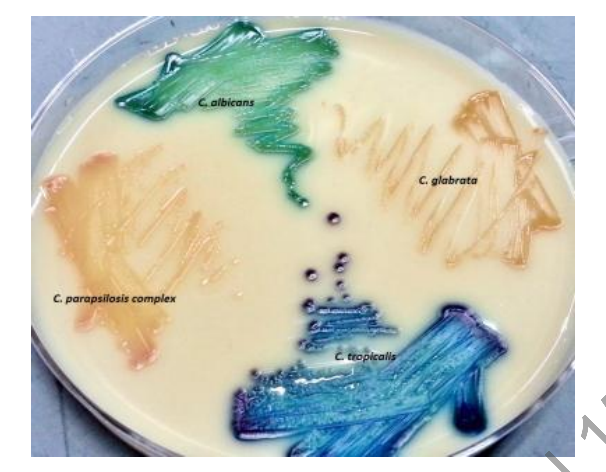
- Use of chromogenic agars to identify commonly encountered Candida species.
- Eg CandiSelect™ 4 (Bio-Rad) can differentiate 4 commonly encountered Candida species
  - C. albicans – appears purple,
  - C. tropicalis, C, glabrata, and C. kruseii – appears turquoise, each with different features (may be difficult to differentiate)
- Eg Brilliance™ Candida agar (Oxoid)
  - C. albicans (green), C. glabrata and C. parapsilosis (biege), C. tropicalis (dark blue)
- Thus plate can be useful for culturing specimens where there can be mixed culture with more than 1 type of Candida species





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Another chromogenic agar – Brilliance™ Candida agar (Oxoid)



Candida albicans : green

Candida glabrata and parapsilosis complex : biege

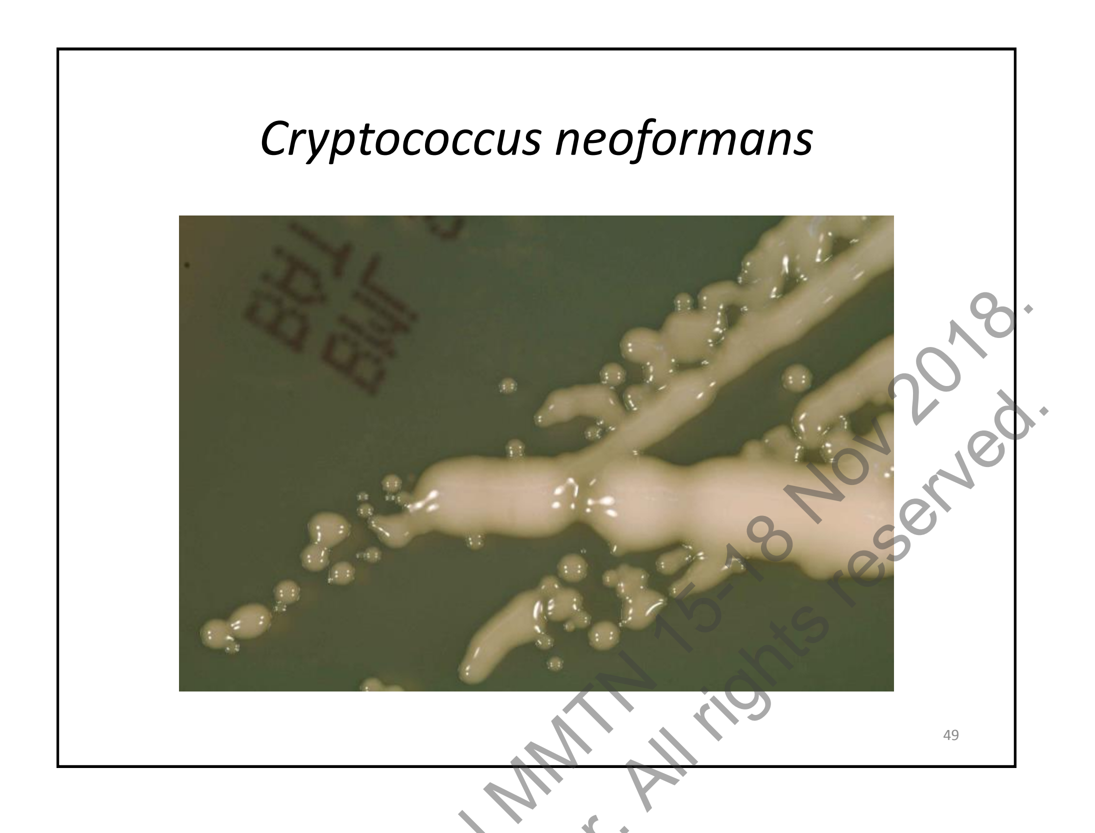
Candida tropicalis : dark blue

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Cryptococcus

- Culture isolates – are slightly slower growing compared with Candida species. Appear more mucoid (because of their capsule)
- Use yeast morphology – see only blastoconidia
- Can do urease test – will be positive. Other yeasts which are urea positive include Trichosporon
- API can identify Cryptococcus species

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Cryptococcus neoformans

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Cryptococcus neoformans
(wet mount)

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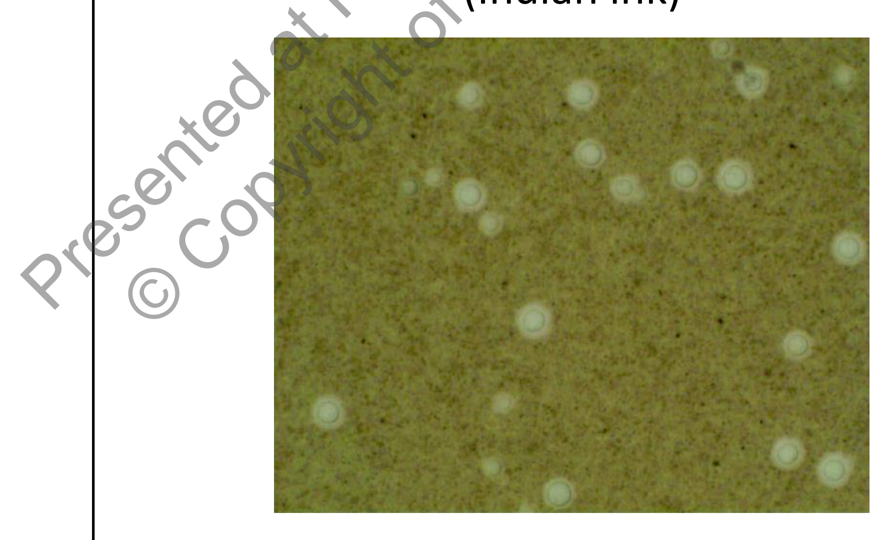
Cryptococcus

- Major yeast infecting humans. More prominent because of AIDS.
- Use of Indian ink on primary specimen like Cerebro-spinal fluid, to see the capsule. Other yeasts will not have similar effect.

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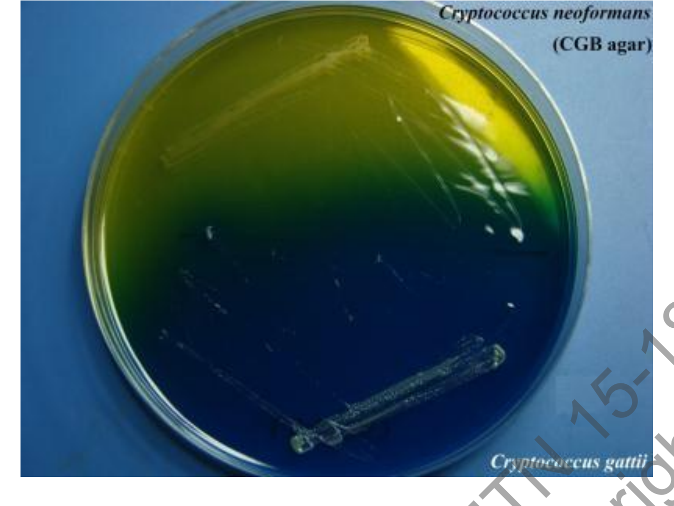
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Cryptococcus neoformans (Indian ink)



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Cryptococcus on canavanine glycine bromthymol blue (CGB) agar



C. neoformans : no change in colour

C. gatti : blue colour

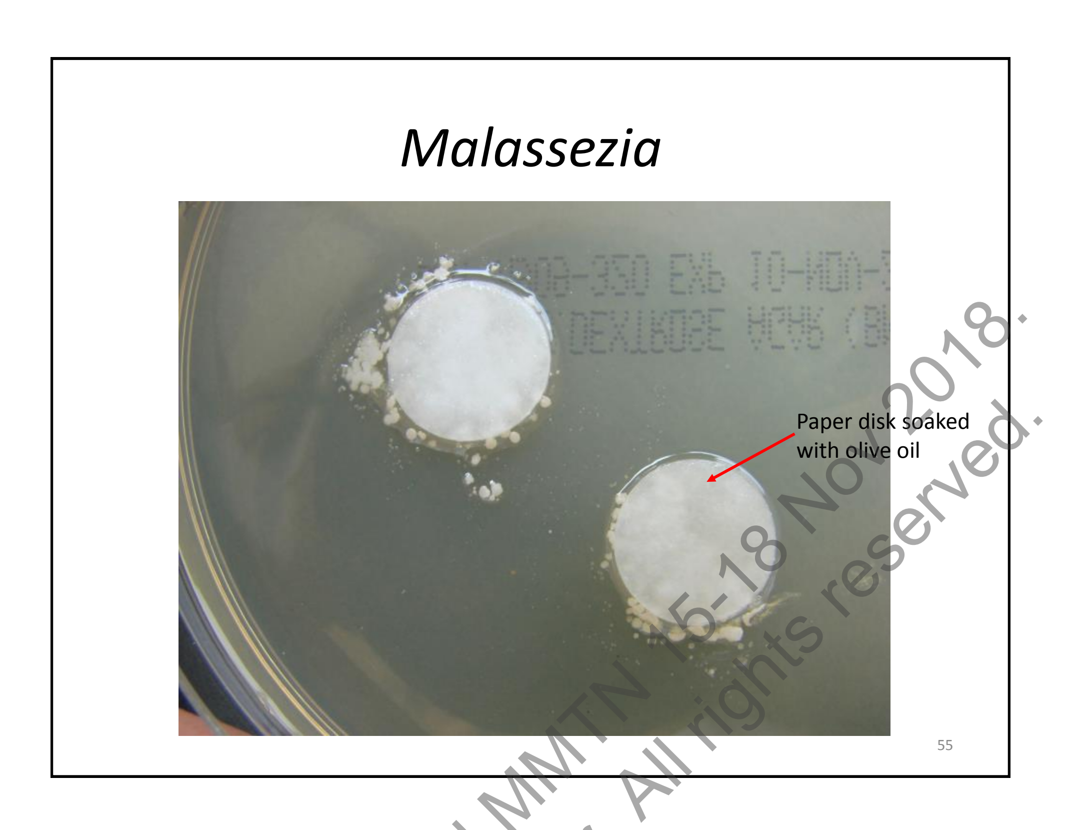
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Malassezia

- Malassezia furfur needs olive oil for growth – put drop onto the SDA, or use a paper disk soaked with olive oil
- Slow growing
- Small white colonies
- Wet mount – shows blastoconidia with budding

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Malassezia



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Malassezia – wet mount

Bottle shaped
broad based
budding

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